UNITED STATES DEPARTMENT OF AGRICULTURE
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POTENCY TEST FOR ERYSIPELAS BACTERIN

1. After reconstitution of the standard as directed, groups of sixteen 16-20 gram mice each are injected subcutaneously with 0.5 ml of the following (or other appropriate) 3-fold dilution in physiological saline: 1:50, 1:150 and 1:450. At the same time, additional groups of the same size and weight are injected subcutaneously with the test bacterin using the same dilutions as for the reference standard.

2. Two weeks after the immunizing injection, the mice are challenged subcutaneously with 0.5 ml. of a culture of E. rhusiopathiae diluted in peptone solution to contain at least 100 MLD in 0.5 ml. At the same time unvaccinated mice are injected subcutaneously with appropriate dilutions of the culture to check on its virulence. The test mice are kept under observation for 7 days.

Bacterin	Dilution No. 1	Dilution No. 2	Dilution No. 3
Standard	16 mice	16 mice	16 mice
Unknown	16 mice	16 mice	16 mice

3. Test for valid assay.

Before the potency of the unknown bacterin can be compared. to the Standard Bacterin the following requirements must be met.

(a) At least two doses of the standard and of the unknown must protect more than 0% and less than 100% of the mice injected. The largest dose of the standard must protect more than 50% and the smallest dose less than 50%.

(b) Subtract the number of survivors in the unknown group from the number of survivors in the Standard group at each dose.

Example	Dilution No. 1	Dilution No. 2	Dilution No. 3
Standard	15/16*	8/16	2/16
Unknown	12/16	9/16	0/16
Difference	3	-1	2

*Survivors/ total injected

Make the middle difference zero by adding the proper figure with the right sign. Thus in this example we add 1. Add this same figure to the other two differences.

Difference No. 1	Difference No. 2	Difference No. 3
3	-1	2
1	1	1
4	0	3

Look in Table I to see if the result is included. If it is included, the assay is valid and the potency of the unknown bacterin can be compared with the Standard. In the above example, this can be done. If the result does not appear in Table I, the test must be repeated.

4. Potency release.

If all the requirements in step 3 are met, proceed as follows:

a) Compare the total number of survivors (25 in example) for the standard with the total number of survivors for the unknown (21 in example) by subtracting unknowns from standard.

b) If the difference is 6 or less, the batch is satisfactory. If the difference is 7 or more, the batch is rejected.

In the example cited, the difference is 4, so the batch is accepted.

If the bacterin does not pass the test as outlined above, the data may be analyzed by any recognized statistical method such as that described by Litchfield and Wilcoxon in THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS Vol. 95, No. 2, June 1949.

D_1*	D_2*	D_3*
-7	0	-1
-7	0	-2
-6	0	0
-6	0	-1
-6	0	-2
-6	0	-3
-5	0	1
-5	0	0
-5	0	-1
-5	0	-2
-5	0	-3
-5	0	-4
-4	0	2
-4	0	1
-4	0	0
-4	0	-1
-4	0	-2
-4	0	-3
-4	0	-4
-4	0	-5
-3	0	3
-3	0	2
-3	0	1
-3	0	0
-3	0	-1
-3	0	-2
-3	0	-3
-3	0	-4
-3	0	-5
-3	0	-6

D_1*	D_2*	D_3*
-2	0	4
-2	0	3
-2	0	2
-2	0	1
-2	0	0
-2	0	-1
-2	0	-2
-2	0	-3
-2	0	-4
-2	0	-5
-2	0	-6
-2	0	-7
-1	0	5
-1	0	4
-1	0	3
-1	0	2
-1	0	1
-1	0	0
-1	0	-1
-1	0	-2
-1	0	-3
-1	0	-4
-1	0	-5
-1	0	-6
-1	0	-7
0	0	6
0	0	5
0	0	4
0	0	3
0	0	2
0	0	1
0	0	0
0	0	-1
0	0	-2
0	0	-3
0	0	-4
0	0	-5
0	0	-6

D_1*	D_2*	D_3*
1	0	7
1	0	6
1	0	5
1	0	4
1	0	3
1	0	2
1	0	1
1	0	0
1	0	-1
1	0	-2
1	0	-3
1	0	-4
1	0	-5
2	0	7
2	0	6
2	0	5
2	0	4
2	0	3
2	0	2
2	0	1
2	0	0
2	0	-1
2	0	-2
2	0	-3
2	0	-4

D_1*	D_2*	D_3*
3	0	6
3	0	5
3	0	4
3	0	3
3	0	2
3	0	1
3	0	0
3	0	-1
3	0	-2
4	0	5
4	0	4
4	0	3
4	0	2
4	0	1
4	0	0
4	0	-1
4	0	-2
5	0	4
5	0	3
5	0	2
5	0	1
5	0	0
5	0	-1
6	0	3
6	0	2
6	0	1
6	0	0
7	0	2
7	0	1

*Difference in number of animals surviving at each dose, corrected to make middle value zero (see page 2 for method).